

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
PATENT EXAMINING OPERATION

Applicant(s): Glen H. ERIKSON et al.

Serial No: 09/664,827

Group Art Unit: 1655

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Examiner: C. Wilder

Att. Docket No.: E1047/20044

Confirmation No.:

For: QUADRUPLIX DNA AND DUPLEX PROBE SYSTEMS

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
Washington, DC 20231

Sir:

I, Richard A. Collins, Ph.D., a citizen of Canada, hereby declare and state:

1. The curriculum vitae attached as Exhibit A accurately reflects my professional credentials. As noted in Exhibit A, I am presently employed as a Professor of Medical Genetics at the University of Toronto.

2. Although I have no financial interest in Ingeneus Corp., I previously acted as a paid consultant for Ingeneus Corp. to supervise and report on the blind studies discussed below. I was paid a one-time fee of \$1000 by Ingeneus Corp. in the year 2000 for that consultation. I have had no other financial dealings with Ingeneus Corp.

3. The following study was conducted under my direct supervision. The purpose of the study was to provide independent verification of the reality and utility of an assay method developed by Ingeneus Corp. The study was designed to blindly test whether the assay could accurately discriminate wild-type from mutant DNA sequences present in duplex targets by the binding of DNA oligonucleotides.

4. Twelve single-stranded oligodeoxynucleotides (DNAs) were

synthesized by ACGT Corp. (Toronto, Ontario, Canada). The DNAs comprised six complementary pairs of sequences: a wild type and a single base substitution mutant in each of three sequence contexts, designated A, B and C, that differed in G+C content from 33% to 73% (see Exhibit B, attached).

5. DNA concentration was estimated, and six pairs of annealed double-stranded DNA were prepared. After several days of refrigeration, eight aliquots of each of the three wild-type and three mutant annealed samples (48 samples in total) were taken, from which subsets were obtained of eight samples from each of the A, B and C series. These 24 samples were labeled A1 through A8, B1 through B8 and C1 through C8. The identity of these samples was not revealed to the workers conducting the testing. Probe and YOYO were added to each sample, each sample was irradiated with a laser and then the fluorescence of each sample was measured to determine whether it contained wild-type or mutant DNA.

6. The assay correctly identified 23 of the 24 samples tested (96% accuracy) as either wild-type or mutant DNA as noted in my report prepared at that time, attached as Exhibit C.

7. Based on my oversight of the foregoing blind tests, my understanding of the assay and my review of the January 18, 2002 DECLARATION UNDER 37 C.F.R. § 1.132 of Jasmine I. Daksis, I agree with Dr. Daksis' conclusion that the triplex assay is a reality and that her data provide evidence that it does not proceed through a strand invasion mechanism.


I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine

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and/or imprisonment under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Date:

Jan. 21/02


Richard A. Collins, Ph.D.

January 2002

CURRICULUM VITAE

Richard A. Collins

BIOGRAPHICAL INFORMATION

Birthdate: February 28, 1954. Regina, Sask. Canada.

Citizenship: Canadian

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EDUCATION:

B.Sc. University of Regina, Regina, Saskatchewan, Canada, 1975.

Ph.D. University of Regina, Regina, Saskatchewan, Canada, 1979

Thesis title: "Mitochondrial Ribosome Assembly in Wild Type and Mutant Strains of *Neurospora crassa*". Supervisor: Dr. Helmut Bertrand.

POST-GRADUATE TRAINING:

09/79-12/83 Department of Biochemistry, St. Louis University School of Medicine.

Supervisor: Dr. Alan Lambowitz

POSITIONS HELD

07/96-present Professor, Molecular and Medical Genetics, University of Toronto

07/91-06/96 Associate Professor, Molecular and Medical Genetics, University of Toronto

07/89-06/91 Associate Professor, Botany, University of Toronto

01/84-06/89 Assistant Professor, Botany, University of Toronto

FELLOWSHIPS AND AWARDS

01/01-01/07 Canada Research Chair in Molecular Biology

07/96-12/00 Senior Scientist Award, Medical Research Council of Canada

08/92-07/02 Fellow, Canadian Institute for Advanced Research Program in Evolutionary Biology

07/91-06/96 Scientist Award, Medical Research Council of Canada

09/79-08/82 Post-doctoral fellowship, Medical Research Council of Canada

09/75-08/79 NSERC post-graduate scholarship

RESEARCH INTERESTS:

RNA-catalyzed reactions; RNA structure and folding; mitochondrial gene expression and evolution; intron structure and RNA splicing; molecular evolution.

SCHOLARLY AND PROFESSIONAL WORK

Publications in Refereed Journals (1995 to present):

Sood, V.D. Yekta, S. and Collins, R.A. (2002). The contribution of 2'-hydroxyls to the cleavage activity of the *Neurospora* VS ribozyme. *Nucl. Acids Res.* accepted.

Sood, V.D. and Collins, R.A. (2001). Functional equivalence of the uridine turn and the hairpin as building blocks of *Neurospora* VS ribozyme structure. *J. Mol. Biol.* 313: 1013-1019.

Hiley, S. and Collins, R.A. (2001). Rapid formation of a solvent-inaccessible core in the *Neurospora* VS ribozyme. *EMBO J.* 20: 5461-5469.

Andersen, A. and Collins, R.A. (2001). Intramolecular base pair rearrangement by the kissing interaction of the *Neurospora* VS ribozyme. *Proc. Nat. Acad. Sci.* 98 7730-7735.

Maguire, J.L. and Collins, R.A. (2001). Effects of cobalt hexammine on folding and self-cleavage of the *Neurospora* VS ribozyme, *J. Mol. Biol.* 309: 45-56.

Tillier, E.R.M. and Collins, R.A. (2000). Replication orientation affects the rate and direction of bacterial gene evolution. *J. Mol. Evol.* 51: 459-463.

Tillier, E.R.M. and Collins, R.A. (2000) Genome rearrangement by replication-directed translocation. *Nature Genetics* 26: 195-197.

Clarke, G., Collins, R.A., Leavitt, B.R., Andrews, D.F., Hayden, M.R., Lumsden, C.J. and McInnes, R.R. (2000). A one-hit model of cell death in inherited neuronal degenerations. *Nature* 406: 195-199; Addendum (2001) 409, 542

Tillier, E.R.M. and Collins, R.A. (2000). The contributions of replication orientation, gene direction and signal sequences to base composition asymmetries in bacterial genomes. *J. Mol. Evol.* 50: 249-257.

Andersen, A. and Collins, R.A. (2000). Rearrangement of a stable RNA secondary structure during VS ribozyme catalysis. *Molecular Cell* 5: 469-478.

Sood, V.D., Beattie, T.L. and Collins, R.A. (1998). Identification of phosphates involved in metal binding and tertiary folding in the core of the *Neurospora* VS ribozyme. *J. Mol. Biol.* 282:741-750.

Olive, J.E. and Collins, R.A. (1998) Spermine switches a *Neurospora* VS ribozyme from slow cis-cleavage to fast trans-cleavage. *Biochemistry* 37: 6476-6484.

Rastogi, T. and Collins, R.A. (1998). Smaller, faster VS ribozymes identify the catalytic core of the *Neurospora* VS ribozyme. *J. Mol. Biol.* 277: 215-224.

Tillier, E.R.M. and Collins, R.A. (1998). High apparent rate of simultaneous compensatory base pair substitution in ribosomal RNA. *Genetics* 148: 1993-2002.

Beattie, T.L. and Collins, (1997). Identification of functional domains in the self-cleaving *Neurospora* VS ribozyme using damage selection. *J. Mol. Biol.* 267: 830-840.

Rastogi, T.R., Beattie, T.L., Olive, J.E. and Collins, R.A. (1996). A long-range pseudoknot is required for activity of the *Neurospora* VS ribozyme. *EMBO J.* 15: 2820-2825.

Olive, J.E., De Abreu, D.M., Rastogi, T., Andersen, A.A., Mittermaier, A.K., Beattie, T.L. and Collins, R.A. (1995). Enhancement of *Neurospora* VS ribozyme cleavage by tuberactinomycin antibiotics. *EMBO J.* 14: 3247-3251.

Beattie, T., Olive, J.E. and Collins, R.A. (1995). A secondary structure model for the self-cleaving region of *Neurospora* VS RNA. *Proc. Nat. Acad. Sci.* 92, 4686-4690.

Kennell, J.C., Saville, B.J., Mohr, S., Kuiper, M.T.R., Sabourin, J.R. Collins, R.A. and Lambowitz, A.M. (1995). The VS catalytic RNA replicates by reverse transcription as a satellite of a retroplasmid. *Genes and Development* 9, 294-303.

Guo, H.C.T. and Collins, R.A. (1995). Efficient trans-cleavage of a stem-loop substrate RNA by a ribozyme derived from *Neurospora* VS RNA. *EMBO J.* 14: 368-378.